Lasers in Surgery and Medicine 18:397–405 (1996)

Effect of Photodynamic Therapy on the Healing of a Rat Skin Flap and Its Implication for Head and Neck Reconstructive Surgery

Alexander Kübler, MD, DMD, Robert K. Finley, III, MD, I. Antonio Born, MD, and Thomas S. Mang, PhD

Department of Oral, Facial, and Maxillary Surgery (A.K.), Department of Pathology (I.A.B.), University of Heidelberg, 69120 Heidelberg, Germany; Department of Surgical Oncology, St. Elizabeth's Regional Cancer Center, Dayton, Ohio 45408 (R.K.F.); Great Lakes Biomedical Laser Center, School of Dental Medicine, University of Buffalo, Buffalo, New York 14214 (T.S.M.)

Background and Objective: **Photodynamic therapy (PDT) may as adjuvant therapy be used to reduce tumor recurrence in the head and neck with surgery, given intraoperatively after resection. A concern with the use of intraoperative PDT is the possible effect on wound healing, especially on the healing of myocutaneous skin flaps, which are widely used to reconstruct defects following resections for head and neck cancer.**

Study Design/Materials and Methods: **A flap, based on the inferior epigastric artery, was prepared in thirty male Lewis rats. Group I did not receive any further treatment but the wound was left open for 20 minutes. Group II was injected with 5mg/kg Photofrin, 48 hours prior to the operation and also did not receive any further treatment. The wound bed and wound borders of group III were treated with 630nm light of different dosages, delivered by an argon dye laser. Animals in group IV received 5mg/kg Photofrin 48 hours prior to the operation and their wound beds were treated with the same light dosages as group III. After the treatment all flaps were replaced into the wound bed and the incisions were closed. Biopsies for histological analysis were taken at several time points; and on day 21, biopsies for wound tensile strength measurements were taken.**

Results: **The wound healing in group I, II, and III appeared normal and there were no differences seen between these groups. Also, the tensile strength did not differ significantly. The flaps of group IV showed serous effusion, epidermal necrosis, and weaker tensile strength (P = .04 and .02 for the light doses of 50 J/sq cm and 75 J/sq cm respectively) at a specific time point.**

Conclusion: **The results of this study demonstrate that PDT given immediately before flap reconstruction will result in delayed wound healing. These results should be considered when contemplating the use of PDT as adjuvant intraoperative therapy for tumor surgery requiring flap reconstruction after ablative surgery.**

Key words: photodynamic therapy, PDT, flap, wound healing, tensile strength

Accepted for publication February 6, 1995.

Address reprint requests to Thomas S. Mang, Ph.D., Great Lakes Biomedical Laser Center and School of Dental Medicine, State University of New York at Buffalo, 112 Squire Hall, 3435 Main St., Buffalo, NY 14214.

INTRODUCTION

Most tumors in the head and neck region are squamous cell carcinomas, which have a propensity for metastasis to cervical lymph nodes and for local recurrence. Although modern techniques of radiotherapy have significantly decreased the rate of local recurrence, advanced-stage head and neck squamous cell carcinomas continue to exhibit a propensity for metastasis to the regional cervical lymph nodes, as well as local recurrence despite multi-modality therapy. For cancers of the head and neck, photodynamic therapy (PDT) has been used for superficial or small tumors with promising results [1–3]. For the treatment of larger tumors, PDT was initially used as a palliative form of therapy. The tumor could be treated with multiple interstitial fiber implantation combined with superficial illumination. In spite of the attempts to uniformly illuminate tumors in advanced cases, curative results are seldom obtained. This may be due to the large mass of these tumors. PDT has therefore been most successfully employed as palliative therapy and as such, the side effects of photosensitivity must be weighed against the potential benefits of extended survival [2,4].

A new application of PDT in advanced tumors of the head and neck may be as intraoperative adjuvant therapy. After surgical resection of the tumor, recurrences emerge from microscopic residual disease. These cells are the target of intraoperative PDT. This combination had already been used for the treatment of mesothelioma, gastrointestinal tumors, and brain tumors [5]. The rationale for the use of localized PDT after surgical resection of the tumor is that recurrences can emerge from microscopic residual disease or field changes which may exist in areas which appear histologically normal. Since the photosensitizer is retained in malignant cells at a higher rate than in most of the surrounding normal tissue, PDT fulfills most of the criteria for selective intraoperative tumor therapy.

Resection of head and neck tumors often results in large, complex defects of bone and soft tissue, skin and mucosal lining of the oral cavity, oropharynx, or hypopharynx. Plastic surgical techniques, including fasciocutaneous, myocutaneous, and myo-osteo-cutaneous vascularized free tissue transfers are necessary for reconstruction.

In this context, the major concern using intraoperative PDT, is the effect which the therapy may have on wound and flap healing after surgery. The status of blood vessels and therefore blood supply is one of the major concerns in flap surgery. The observed shut down of blood vessels [6], as has been demonstrated in animal models and which may be caused by PDT, may interfere with healing and survival of flaps in the wound beds. Therefore, the vascular effect of PDT and its implication in flap healing must be considered prior to adjuvant use of PDT in head and neck surgery.

Preliminary studies in animal models have demonstrated that PDT has significant impact on wound healing including delay of early granulation tissue formation, delay in re-epithelization, and necrosis of muscle and mucosal tissues [7,8]. It is not clear whether this interference in wound healing would contraindicate the adjuvant use of PDT when flap reconstruction is required. Reconstructed areas in the head and neck are sometimes exposed to high mechanical forces which make wound tensile strength an important consideration for evaluating healing in the head and neck region.

MATERIALS AND METHODS

Thirty male Lewis rats (Charles River) weighing 250–300g were used. The protocol was approved by the local Animal Use Committee. Rats were housed in an air-conditioned room and fed with rat chow and water ad libitum. The animals were kept in wire mesh cages without bedding, so the wounds remained clean, though not sterile.

The rats were divided into four groups consisting of five (group I), three (group II), 11 (group III), and 11 (group IV) animals. One rat in group IV died during anesthesia. The groups were assigned treatments according to the following regimen: *group I*—controls—no Photofrin (QLT, Vancouver, British Columbia, Canada)—no light; *group II*—Photofrin only (5mg/kg); *group III*—no Photofrin—light (630nm) alone; *group IV*—complete PDT—5mg/kg Photofrin and light (630nm). The rats of group II and IV were injected intraperitoneal (i.p.) with 5mg/kg Photofrin, 48 hours prior to the operation.

After adequate anesthetization with 35 mg/kg pentobarbital i.p., the abdomen and groin area of the animals were shaved using a razor followed by the use of a depilation cream. After aseptic preparation, a rectangular standard groin flap [3 × 2cm (longitudinal × lateral axis)], stemmed at the superficial epigastrical vessel was surgically raised. The incision penetrated down

to, but did not include the skeletal muscle. The flap was folded back and covered with gauze and sterile black paper. The flap and the wound bed were moistened repeatedly with 0.9% sodium chloride during the entire experiment.

The animals of group I (control) and II (Photofrin control) did not receive any further treatment. The flap was kept in the raised position for twenty minutes before it was placed back followed by wound closure using 9mm autoclips (Clay Adams, Lincoln Park, NJ). The wound beds in group III (light control) and IV (complete PDT) were treated with various doses of 630nm light (spot diameter 3.4cm; power density 75mW/cm^2) delivered by an argon dye laser (Spectraphysics, Mountain View, CA). In group III and IV, three rats were treated with 25J/cm^2, four rats with 50J/cm^2, and four rats with 75J/cm^2. Only the wound bed and borders were exposed to the light, as the flap was folded back and covered with gauze and black paper. Also the superficial epigastric artery, where the flap was based, was covered, so light or PDT effect could not cause any direct damage to the nutrient blood vessel or the flap itself. Following laser treatment, the wound was kept open for 20 minutes (including laser treatment time) prior to closing using 9mm autoclips. The flap and the wound were checked every other day for wound healing problems. On day 7, all autoclips were removed.

Histological Analysis

Biopsies were taken in a non-traumatic fashion under general anesthesia after four hours, twenty-four hours, seven days, fourteen days, and twenty-one days post-treatment. All biopsies comprised parts of the flap, the wound, and the surrounding skin. The specimens were fixed in 10% buffered formalin, embedded for paraffin sectioning, cut in standard fashion, and stained with hematoxylin-eosin. Six microscopic slides were made from each sample, with all sections cut perpendicular to the wound edges.

A pathologist, blinded with reference to the treatment groups, examined the histological slides for differences in wound healing between and within the groups.

Tensile Strength Measurements

On day 21, the rats were anesthetized again using 35mg/kg pentobarbital i.p. A skin sample size 2.5 × 0.3cm perpendicular to the axis of the wound edge was excised for tensile strength measurements. Each test sample contained the wound area in its mid-section. The samples were stored in Ringer's solution at 7°C for up to 12 hours prior to the tension test. After measuring the width and thickness of the skin sample at the wound area, the sample was placed in the grips of a tensile strength instrument (Columbia Lab, Buffalo, NY). The specimen was then distended with a constant speed of 21.2 mm/min up to the point of rupture. During the distraction test, the allied forces were recorded continuously. The tensile strength of the sample was calculated by dividing the recorded breaking strength (grams) by the cross-sectional area of the sample (width × thickness [in mm^2]). All measurements were made in a blind fashion.

Statistics

All results were calculated by the Mann-Whitney test.

RESULTS

As a prelude to the actual experiment, we intentionally severed the supplying blood vessels to determine the consequence on wound healing. Within 2 minutes after dissection of the blood vessels, the flap became blue (i.e., cyanotic) due to insufficient blood supply. As a result of this cyanotic reaction, we could visually evaluate the intactness of the supplying epigastric artery during and after the operation. Since no such reaction was visible during the experiment, we could be confident that the nutrient blood vessels were kept intact for all flaps.

Macroscopically, the wound healing in groups I (control), II (Photofin control), and III (light control) did not differ significantly. All flaps healed without any signs of problems or inflammation. We could not find any significant differences within or between the groups. When the last biopsies were taken on day 21, it was often difficult to identify the scar. Wound healing differed significantly in the PDT treated rats (group IV). Problems were observed within 24 hours post-treatment. In all rats given complete PDT (drug + light), regardless of light dose, a significant hemorrhagic-serous effusion developed below the flap by the 24-hour biopsy time-point. The effusion was drained in the process of taking the biopsy. There was no further effusion observed on day 7.

During the first few days following the PDT treatment, the color of the flaps changed. Within 3 to 4 days the flaps became slightly yellow/grey. This was followed by necrosis and scab formation

of the epidermis. The necrosis comprised only the epidermis. When the scab was removed by forceps, new epidermis formation underneath was observed. Necrosis of the epidermis seemed to occur independent of light dose. Within 21 days, all erosions and scabs resolved, with only slight residual scarring remaining. Two rats treated with 50J/cm^2 and 75J/cm^2 showed only localized erosions and scab formation at the wound border, whereas the flaps themselves were only slightly affected.

Histological Analysis

The biopsies of group I served as controls for normal wound healing. The wound healing in groups II and III appeared to be similar to that of group I for all time-points. In contrast, there were notable differences in wound healing between these groups and the PDT group (group IV). The 4-hour biopsies of group I, II, and III demonstrated an intense margination of inflammatory cells and extravasation of red blood cells along the incision line and in the deep vascular plexus. In addition, a moderate vasodilatation and fibrin exudate appeared (Fig. 1). In group IV (PDT-group), all reactions, primarily the margination of inflammatory cells and extravasation of red blood cells, were much less intense, whereas in this group serum extravasation was distinguishable (Fig. 2). After 24 hours, the biopsies from all groups showed local ulcerations and inflammatory reaction of the epidermis. This was probably a reaction to shaving and application of depilation cream immediately before the operation. In group I, II, and III, the first signs of granulation tissue formation along the incision line could be seen. In group IV, the inflammatory reaction at 24 hours became more intense, while a complete lack of granulation tissue formation was noted. This lack of granulation tissue produces a weaker incision line, along with a partly gaping wound. At this time the epidermis of the skin in the treatment area appeared to be slightly atrophic.

On day 7, the biopsies of group I, II, and III showed only some residual chronic inflammatory reactions in the deep vascular plexus (Fig. 3). In these samples, the former incision line was connected by parallel collagen fibers and some residual granulation tissue. The epidermis displayed a hyperkeratotic and parakeratotic reaction. Compared to this, the inflammatory reaction in the PDT group (IV) was still active along the incision line as well as in the deep plexus (Fig. 4). There was also a weaker incision line with occasional erythrocyte extravasation. Epidermal atrophy was still observable.

After 14 days, all biopsies in group I, II, and III showed a nearly completely healed scar with only occasional chronic inflammatory infiltration in the deep plexus. In contrast, in the PDT group there was chronic inflammatory infiltration along the incision line and especially in the deep plexus. The scar at the former incision line was rather wide and pyramidal-shaped compared to the other groups.

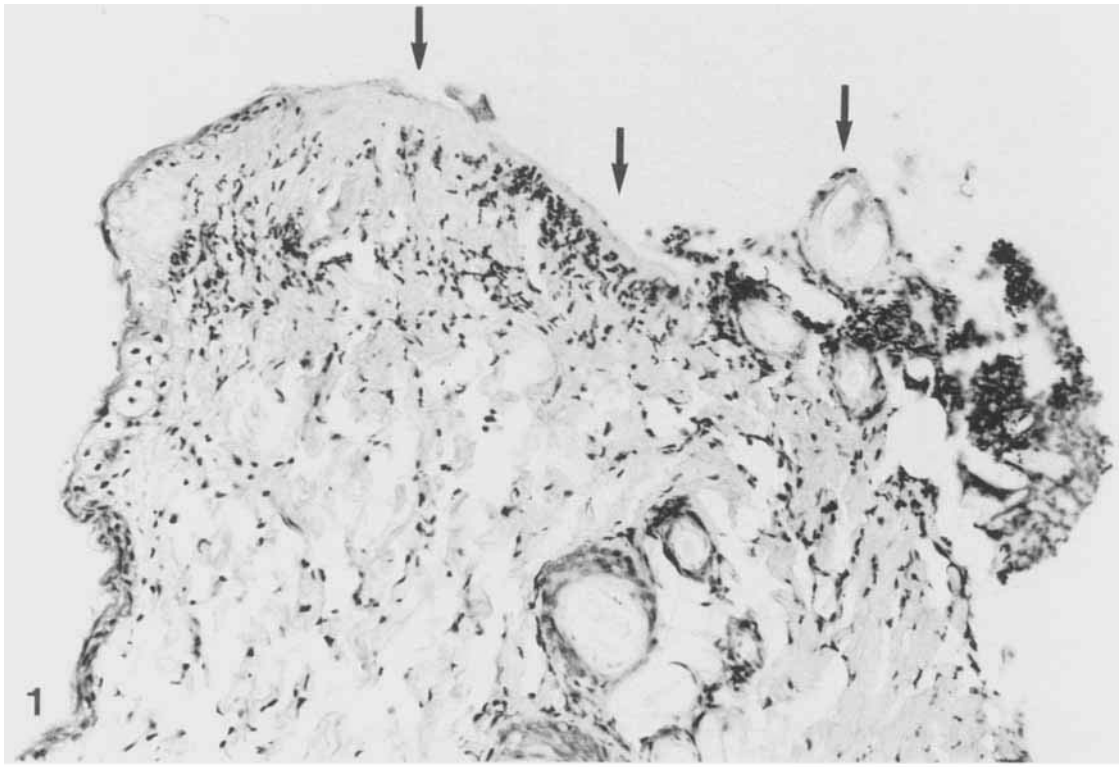
After 21 days, the incision in group I, II, and III was completely healed with scar formation and some occasional local chronic inflammatory infiltrations. This was produced by a foreign body reaction probably due to transferred hair follicles by the operative technique into the deep plexus which remained within the old incision line. In group IV, there was a persistence of granulation tissue and inflammatory infiltrates. The epidermis of the skin in the treated area appeared to be atrophic.

Tensile Strength Measurements

The mean value for tensile strength of the control group (I) was 93.7g/cm^2 (23.7 SD) and the mean value of the Photofrin group (II) was 102.1g/cm^2 (31.0 SD) (Fig. 5). There was no significant difference between these groups. Mean values for the light treatment group (III) were 104.6g/cm^2 for the animals treated with 25J/cm^2, 96.6g/cm^2 at 50J/cm^2, and 126.7g/cm^2 at 75J/cm^2. There was no significant difference between the control group (I) and the light treatment groups (III), although the difference between the control group and the 75J/cm^2 light treatment group approached significance ($P \leq 0.06$). The mean tensile strength measurements for the PDT group (IV) were 81.2g/cm^2 for 25J/cm^2, 58.7g/cm^2 for 50J/cm^2, and 56.5g/cm^2 for 75J/cm^2 (Fig. 1). The mean values for the tensile strength of the PDT groups treated with 50J/cm^2 and 75J/cm^2 were significantly lower than the control group (I) ($P \leq 0.04$ and $P \leq 0.02$). Furthermore, the difference between the 75J/cm^2 PDT group and the 75J/cm^2 light alone group was significant ($P \leq 0.02$).

DISCUSSION

In this study, several different methods have been used to evaluate the effect of PDT on the wound healing of a pedicle skin flap. Since this type of procedure is widely used in head and neck cancer surgery, it is important to study the im-



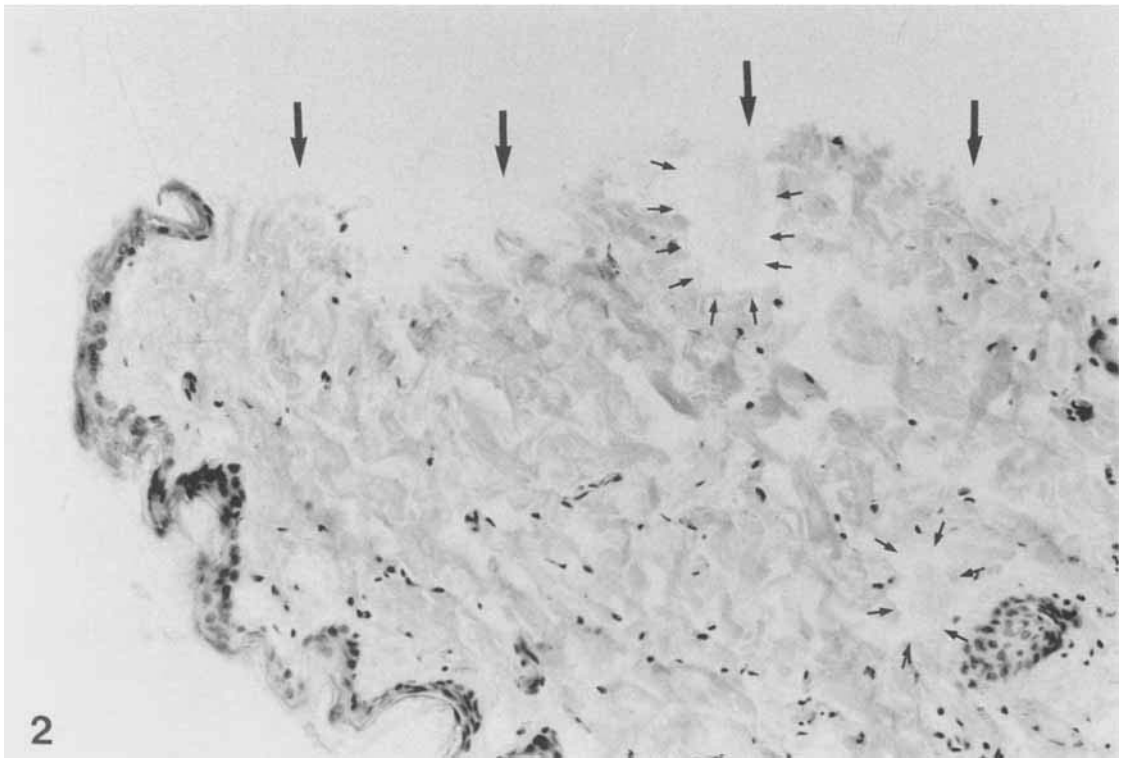


Fig. 1. Group I, rat skin biopsy 4 hours after treatment (H&E; ×25 original). Intense margination of inflammatory cells along the incision line (arrows).

Fig. 2. Group IV, rat skin biopsy 4 hours post-PDT (H&E; ×25 original). The incision line (large arrows) demonstrates a complete lack of inflammatory cells and a discreet serum extravasation (small arrows).

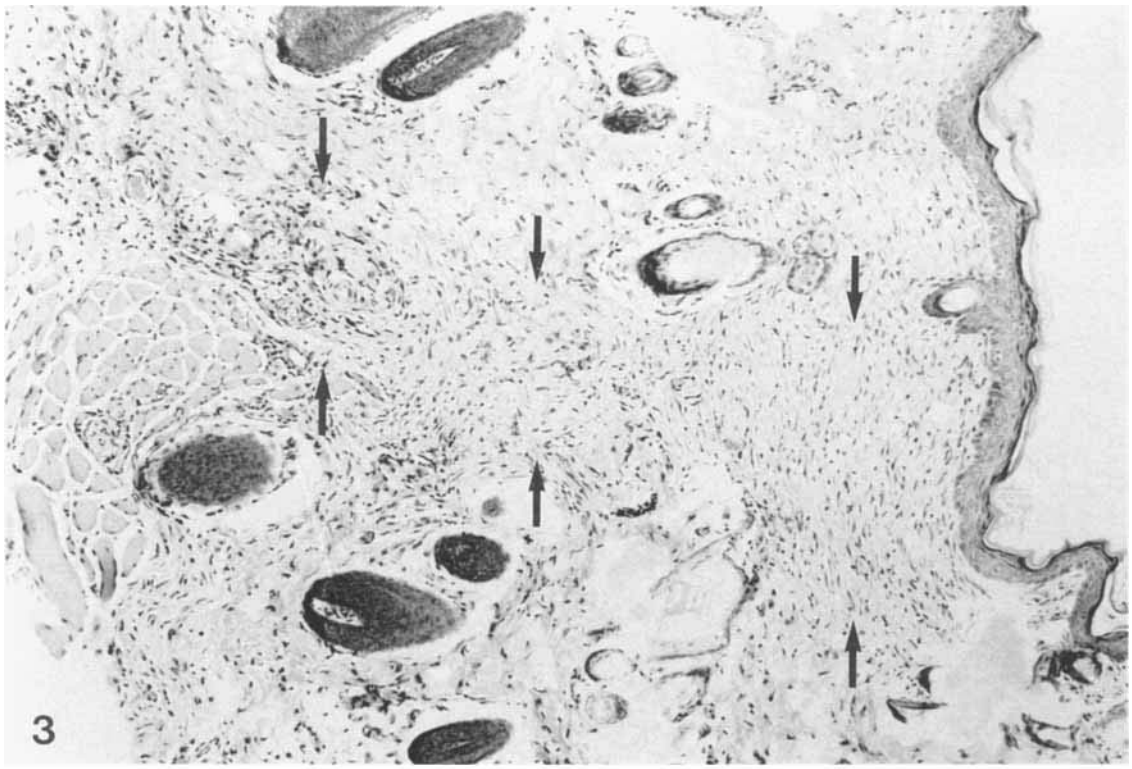


Fig. 3. Group I, rat skin biopsy 7 days post-treatment (H&E; ×10 original). The former incision line (arrows) is connected by parallel collagen fibers and some residual granulation tissue in the deep plexus.

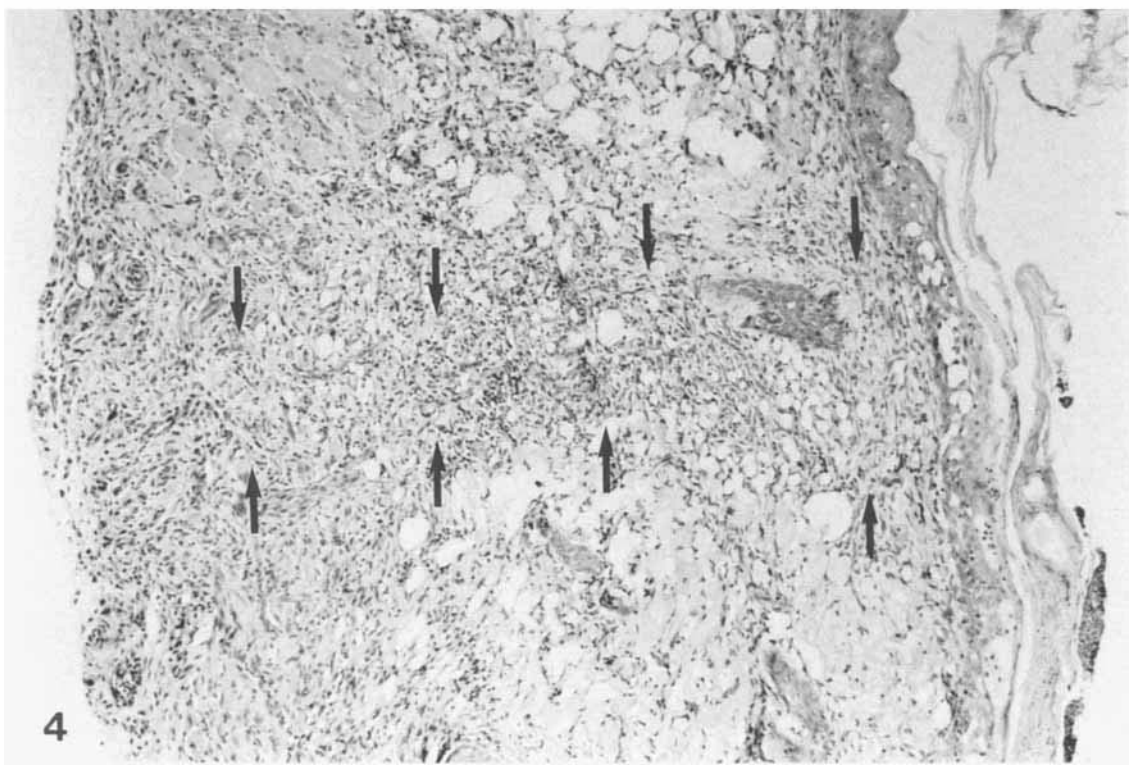


Fig. 4. Group IV, rat skin biopsy 7 days post-PDT (H&E; ×10 original). The former incision line (arrows) is closed by granulation tissue with a still rather active inflammatory reaction. The epidermis shows an atrophic reaction with vacuolic degeneration and inflammatory cells.

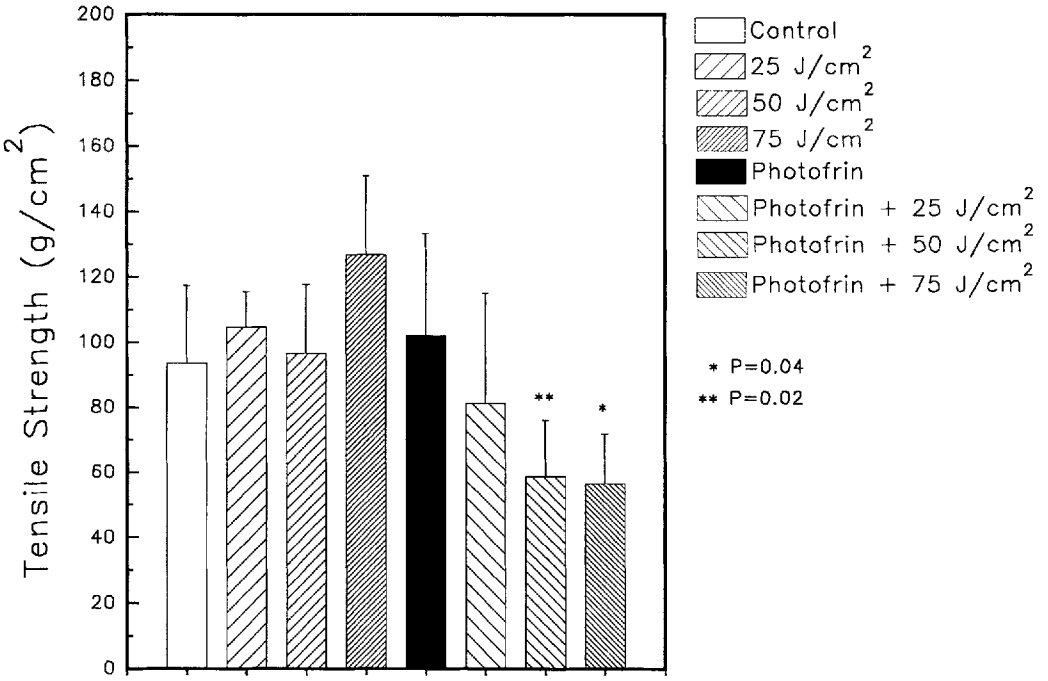


Fig. 5. Wound tensile strength (g/cm^2) of the different treatment groups twenty-one days post-treatment.

pact of PDT on flap healing. In this study, only the wound and the surrounding skin were exposed to laser light as opposed to the flap and the nutrient vessel which were covered during treatment. This procedure was chosen in order to simulate the technique which may be used for intraoperative PDT treatment in head and neck surgery. Head and neck reconstructive surgery often involves a very complex wound healing system, where skin, subcutaneous tissue, bone, and possibly lining of the oral cavity, oropharynx, and hypopharynx is involved. This model simply addresses the effect of PDT on the wound healing of a pedicled rat groin flap and does not address the more complex issues encountered in wound healing in the head and neck surgery patient. However, it increases our understanding of the effect of PDT on wound healing in the situation where a pedicled flap has been raised and the recipient bed is treated with PDT. This situation will be encountered, though in a more complex form, if this technology is to be used as an intraoperative adjuvant therapy for head and neck carcinomas.

The healing and survival of a flap in its new bed depends on the blood supply of the flap and also that of the recipient bed, which is controlled by several factors. First is the size of the flap: in several studies which dealt with the flap size, a defined correlation between length and width has been found as a requirement for the survival of the flap. In other words, the flap should not be too long for its base, otherwise the distal portion will become necrotic. The flap size chosen in this study is rather small. Since the control group, light group, and photofrin group procedures were successful, this demonstrates that the flap size used seems to have no impact on the flap survival rate. Several previous studies have demonstrated that the blood supply of a flap during the first 2 to 4 days after an operation depends upon the vessels which stem from the flap base. During this time, new vascular junctions between the flap, the surrounding wound area and the flap bed will be formed [9]. Reduction or delay of the vessel formation may result in necrosis of the flap. Until new vessel connections have been formed, the blood supply in the distal part of the flap can drop 7% compared to normal skin [10,11]. During the time of reduced blood flow, the flap does not become necrotic due to the fact that skin can survive with a rather low blood flow for a limited time (2 to 8ml/min per 100g or 20% of normal skin) [12]. The reduced blood flow causes ischemia in the most distal areas of the flap, which serves as an important stimulant for flap revascularization [13,14]. If the reduced blood supply persists too long, irreparable damage and flap necrosis will occur.

After PDT, several vascular effects such as platelet aggregation, vasoconstriction as well as vasodilatation, followed by complete blood stasis and hemorrhage have been observed [6]. The extent of this effect seems to correlate with the plasma level of the photosensitizer [15]. Reed et al. reported that the sensitivity of blood vessels from normal skin and tumor to PDT is the same, provided that the circulating level of photosensitizer is equivalent [16]. Sandblom also observed that decreased microcirculation produced by hypovolemia or dehydration reduced the breaking strengths of wounds [17]. The histology of biopsies taken in this study did not show any remarkable vascular shut down, but the PDT group (IV) had a significant delay in inflammatory reaction and wound healing. The development of effusion within 24 hours, underneath the flap of the PDT treated wounds was evidence for vascular leakage. There was histological correlation of serum extravasation into the tissue. Similar observations have been reported by Hayata et al. who describe a large amount of secretion in the bronchi after endoscopic PDT treatment of lung cancer [18].

Although initial difficulties in wound healing after PDT were observed within the first few days, after 21 days there were no scars or residual areas of epidermal necrosis. This demonstrated that the effect of PDT was not severe enough to cause total loss of the flap. Still, PDT did produce a delay in wound healing reflected by the weaker tensile strength at 21 days. The reported epidermal necrosis in the PDT group does not correspond with the skin necrosis after transcutaneous PDT treatment of dermal or subdermal tumors. In the case of skin tumors, the normal skin is generally included in the treatment field, whereas in our study the flap was folded back and covered with gauze and black paper. The epidermal necrosis of the flap has to be an indirect effect of PDT on the flap epidermis possibly caused by the effect of PDT on the recipient bed. Only the atrophy of the epidermis in the treated areas reflected a direct epidermal effect of PDT.

The tendency for an increase in the tensile strength in the light-alone treatment group (group III) reflects the effect of light on the promotion of wound healing. There have been several publications regarding the enhancement of wound healing by red light. The light dosages used in those studies were lower than those used for PDT. Nevertheless several studies have shown a positive effect of light on wound healing [19–21].

The results of our study demonstrate a detrimental effect of PDT on wound healing in the rat groin flap model. This effect is evidenced in epidermal necrosis of the flap and in decreased wound tensile strength. These results suggest therefore, that the use of PDT in combination with flap surgery can be accomplished with caution. Even if the flap is not illuminated directly, it should be considered, that the PDT effect on the wound bed and at the wound borders could possibly lead to healing delays. Skin exposure to light in the operating room should be minimized. It is also suggested from this pre-clinical study that adequate closed suction drains should be placed beneath the flap and wound bed to provide drainage and prevent serum effusion. Suture removal should probably be delayed due to effects on tensile strength.

ACKNOWLEDGMENTS

The authors thank Dr. Charles Liebow for his comments and expertise in the review and revision of this manuscript, and Dr. Thomas J. Dougherty for his encouragement. This study was supported in part by a grant of the Deutsche Forschungsgemeinschaft and in part by NIH grant 5R01CA47299-02.

REFERENCES

1. Monnier Ph, Fontolliet Ch, Wagnieres G, Braichotte D, Van den Bergh H. Further appraisal of PDI and PDT of early squamous cell carcinomas of the pharynx, oesophagus and bronchi. In: Spinelli P, Dal Fante M, Marchesini R, eds. "Photodynamic Therapy and Biomedical Lasers." Elsevier Science Publishers, Amsterdam, 1992, 2:7–14.
2. Wenig HL, Kurtzman DM, Grossweiner LI, Mafee MF, Harris DH, Lobraico RV, Prycz RA, Appelbaum EL. Photodynamic therapy in the treatment of squamous cell carcinoma of the head and neck. Arch Otolaryngol Head Neck Surg 1990; 116:1267–1270.
3. Feyh J, Goetz A, Müller W, Königsberger R, Kastenbauer E. Photodynamic therapy in head and neck surgery. Photochem Photobiol 1990; 7:353–358.
4. Gluckman JL. Hematoporphyrin photodynamic therapy: is there truly a future in head and neck oncology? Reflection on a 5-year experience. Laryngoscope 1991; 101:36–42.
5. Pass HI. Photodynamic therapy on oncology: mechanisms and clinical use [review]. J Natl Cancer Inst 1993; 85: 443–456.
6. Henderson BW, Dougherty TJ. How does photodynamic therapy work? Photochem Photobiol 1992; 55:145–157.
7. Stern SJ, Thomson S, Small S, Jacques S. Photodynamic therapy with chloroaluminium-sulfonated phthalocya-

nine. Arch Otolaryngol Head Neck Surg 1990; 116:1259–1266.
8. Jefferis AF, Chevretton EB, Berenbaum MC. Muscle damage and recovery in the rabbit tongue following photodynamic therapy with hematoporphyrin derivate. Acta Otolaryngol (Stockh) 1991; 111:153–160.
9. Young CM. The revascularization of pedicle skin flaps in pigs: a functional and morphologic study. Plast Reconstr Surg 1982; 70:455–464.
10. Quirinia A, Jensen T, Viidik A. Ischemia in wound healing. I: design of a flap model—changes in blood flow. Scand J Plast Reconstr Hand Surg 1992; 26:21–28.
11. Guba AM. Study of the delay phenomen in axial pattern flap. Plast Reconstr Surg 1979; 63:550–557.
12. Myers B. Understanding flap necrosis [editorial]. Plast Reconstr Surg 1986; May:813–814.
13. Myers MB, Cherry G. Mechanism of the delay phenomenon. Plast Reconstr Surg 1969; 44:52–57.
14. Palmer B, Jurell G, Norberg KA. The blood flow in experimental skin flaps in rats studied by means of the ^{133}Xenon clearance method. Scand J Plast Reconstr Surg 1972; 6:6–12.
15. Bellnier DA, Dougherty TJ. The time course of cutaneous porphyrin photosensitization in the murine ear. Photochem Photobiol 1989; 49:369–372.
16. Reed MWR, Wieman TJ, Schuschke DA, Tseng MT, Miller FN. A comparison of photodynamic therapy on normal and tumor blood vessels in the rat microcirculation. Radiat Res 1989; 119:542–552.
17. Sandblom P. The tensile strength of healing wounds. An experimental study. Acta Chir Scand 1944; Suppl 89.
18. Hayata Y, Kato H, Konaka C, Ono J, Takizawa N. Hematoporphyrin derivative and laser photoradiation in the treatment of lung cancer. Chest 1982; 81:269–277.
19. Kana JS, Hutschenreiter G, Haina D, Waidelich W. Effect of low-power density laser radiation on healing of open skin wounds in rats. Arch Surg 1981; 116:293–296.
20. Lyons RF, Abergel RP, White RA, Dwyer RM, Castel JC, Uitto J. Biostimulation of wound healing in vivo by a helium-neon laser. Ann Plast Surg 1987; 18:47–50.
21. Surinchak JS, Alago ML, Bellamy RF, Stuck BE, Belkin M. Effect of low-level energy lasers on the healing of full-thickness skin defects. Lasers Surg Med 1983; 2:267–274.